20 Toxicological Concerns Related to Nanoscale Drug Delivery Systems

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20.1 INTRODUCTION

Nanotechnology is an emerging science of precise manipulation of atomic or molecular structures of materials at the nanometer level with unique properties and applications (Miyazaki and Islam 2007; Bakand et al. 2012). Over the past few years, there has been an increasing toxicological concern regarding the safety of the developed nanosystems. Assuming biocompatibility and biodegradability of most of the materials, the toxicity issues caused by them when formulated into nanoparticles (NPs) are usually neglected by the scientific community. Only a few studies approach the

toxicity of the nanosystems. However, nanomaterials safety data are limited to arrive at an overall picture of material-specific risks. The NP characteristics such as size, charge, and surface properties could influence their pharmacokinetics after oral administration.

In health-care delivery, the drug availability is a secondary reason rather than the therapeutic effect. For a therapeutic system to be successful, it is desirable that the required drug should reach the site of action without any undesirable interactions and be available for a sufficient period of time. On the contrary, there are many drug candidates that show low bioefficiency due to different factors such as undesirable interactions, immature degradation, and insufficient capability of tissue penetration. To overcome these problems and monitor therapeutic actions, novel drug delivery systems are being introduced to discharge the drug molecules at the desired site for sufficient duration.

Despite numerous benefits, the potential dangers of NP exposure cannot be ignored. The nanotoxicological responses of NPs are primarily observed on adult healthy animals. Therefore, their effects on susceptible populations are not well known. The perturbations of physiological structures and functions in susceptible populations may exhibit unusual pharmacokinetic profiles of the NPs.

The safety concern regarding the exposure of NPs in humans may restrict the wider application of these promising nanomaterials. NPs may enter the human body via respiratory pathways, digestive tract, intravenous (IV) injection, implantation, and other routes (Arora et al. 2012; Araújo et al. 2015). After absorption, the NPs are carried to distal organs by the bloodstream and the lymphatic system (Oberdörster et al. 2005). During this process, they interact with biological molecules and perturb physiological systems. Some ingested or absorbed NPs are eliminated and the rest remain in the body for a long time. The unexpected invasion of the physiological systems by the NPs disturbs normal cell signaling, cell and organ functions, and may even cause pathological disorders.

Nanotechnology offers some obvious benefits, including better treatment efficacy, specific localization, reduction of dosage regimens, and dose-related side effects (Sahoo and Labhasetwar 2003; Svenson and Tomalia 2005). Other nanostructures are also assumed for use in diagnostics known as nanodevices. However, the nanomaterials have a large surface area to volume ratio, which enables them to alter biological properties as compared to the parent molecule (Williams 2008). In the present scenario, a number of nanoscale drug delivery applications have been attempted for the treatment of cancer, central nervous system (CNS) disorders, and so on. Liposomes, nanoshells, nanotubes, dendrimers, NPs, nanospheres, aquasomes, and solid lipid NPs (SLNs) are among the different nanostructures, commonly known as nanocarriers (Moghimi et al. 2005). Nanomaterials can be generated from different parent materials in different shapes such as spheres, rods, wires, and tubes (Liu 2006). Apart from these differences, similar toxicological profiles are expected.

Further, the polymers are usually required for the fabrication of drug delivery systems. The polymers can help in achieving desired pharmacotherapy by stabilizing the proposed medication during production. Further, the structural manipulation of the polymers is required in order to fabricate different forms, namely, films, microspheres, monoliths, NPs, and polymeric prodrugs (Amsden and Cheng 1995;

Kim et al. 2009), and control the release of drug at the target organ/tissues (Vilar et al. 2012). However, the fabrication strategy depends on the intended route of administration, pharmacokinetics, and the drug efficacy. The design of polymeric carriers intended for delivery via different routes may raise some questions regarding the fate of carrier materials, that is, accumulation or elimination, in the body after completion of drug release. Indeed, the biodegradability of polymers is one of the most important regulatory issues since this property of the polymers determines their consequent removal from the body.

In this section, several nanosystems are described with special reference to their toxicological concerns. A number of NPs based on different types of materials such as polymers, lipids, carbon, and metals are reviewed. The different nanosystems may precipitate toxicity by perturbing different physiological systems as revealed by animal studies. Hence, the factors that contribute toxicity to the nanomaterials are also discussed herein.

20.2 FACTORS AFFECTING TOXICITY OF NANOCARRIERS

A generalization of nanomaterial-related toxicity is difficult due to a large difference in physicochemical properties between the materials and their products. The biokinetics are found to be affected by different physicochemical properties of the nanocarriers that encompass particle size, morphology, surface area, chemical reactivity, surface charge, and state of aggregation (Lockman et al. 2004; Radomski et al. 2005; Hardman 2006; Jiang et al. 2008; Sonavane et al. 2008). Undoubtedly, it is of utmost priority to figure out the different forms of toxicological phenomena and understand the effects caused by the novel nanocarriers, nanodevices, or by occupational exposure of the nanostructures. The biological effects may be beneficial or harmful as supported by very limited data. The physicochemical parameters must be cautiously manipulated before the design of various nanostructures using polymeric materials. Research on the potential health risks on exposure to NPs lags behind the rapid development of nanotechnology. In general, the biological impacts and toxicity of NPs are functions of multiple parameters, and therefore the various characteristics must be addressed for evaluating toxicity of the nanomaterials.

20.2.1 Particle Morphology

The biodistribution, biological fate, toxicity, as well as drug-targeting capacity depend on size and size distribution of NPs. De Jong et al. (2008) conducted an experiment with IV gold NPs (AuNPs; 10, 50, 100, and 250 nm) to investigate the impact of particle size on the biodistribution in mice. Irrespective of sizes, the majority of the Au was present in liver and spleen after 24 h. A clear distinction was evident between the distribution of the 10-nm particles and the larger particles. The smaller particles accumulated in almost all vital organs, whereas the larger ones were only detectable in blood, liver, and spleen. The results demonstrated size-dependent tissue distribution of AuNPs. The smaller size NPs showed the most widespread organ distribution. The discrepancy in tissue distribution pattern of different sized particles was likely to induce damage of varying degrees to tissues or organs.

Usually, the adsorption of opsonin increases the recognition of foreign materials for phagocytosis and cause rapid clearance of the circulating NPs. The polymer particles of hydrophilic surface (<100 nm) show prolonged circulation by delaying opsonization (Alexis et al. 2008; Bertrand and Leroux 2012). The particle size may have an impact on the entry mechanism into target cells and phagocytes. NPs that are <200 nm are internalized via clathrin-coated pits, whereas 500-nm particles are internalized via caveolae-meditated endocytosis (Rejman et al. 2004).

The NPs smaller than serum albumin (~40–50 kDa or a diameter of ≤4–6 nm) are eliminated primarily through the kidneys. The particles or aggregates (>10 μm) are passively entrapped within the lung capillaries. The particles greater than 3 μm size are transiently entrapped and are subsequently moved from lung to the liver (Deshmukh et al. 2012). Particles that lie in the range of 3–6 μm accumulate in the liver and spleen. Thus, the particle size determines the deposition sites in tissues. It is hypothesized that bulk materials (>1 μm) that are relatively inert may become toxic when their size is reduced to the nanoscale level. This could be attributed to greater biodistribution at high surface/volume ratio, and the ability of nanomaterials to traverse cell barriers. The surface of materials interacts with other nanoscale biological molecules such as deoxyribonucleic acid (DNA), proteins, and cell membranes (Xia et al. 2009). The interaction of molecular oxygen and electron donor or acceptor groups on the particle surface generates either superoxide or hydrogen peroxide. Both species can oxidize other compounds through an electron transfer mechanism (Semete et al. 2010a,b). The propagation of reactive oxygen species (ROS) is associated with nanometric size of the materials, and therefore constitutes a mechanism of generating potential toxicity (Nel et al. 2006).

PEGylated AuNPs (13 nm in size) demonstrated long circulating half-lives for ~1 week. They accumulated within the liver and spleen over the course of one week. The sequestration of AuNPs within lysosomes of Kupffer cells and spleen macrophages resulted in acute hepatic inflammation and apoptosis in mice (Cho et al. 2009, 2010).

In its extended conformation, polyethylene glycol (PEG) provides steric hindrance to the adsorption of serum proteins on the surface of NPs, thus delaying phagocytosis by macrophages, and rendering them long circulating property (Owens and Peppas 2006).

In addition to particle size, Chithrani et al. (2006) investigated the impacts of morphology on cellular intake of AuNPs. In spite of their similar dimensions (74 and 14 nm), the uptake of nanorods was slower than the spherical particles in HeLa cells. The ellipsoid particles are more readily engulfed by macrophages than that of spherical particles (Sharma et al. 2010). However, NPs with high aspect ratios (tubular shape vs. spherical) resist uptake by macrophages because of high curvature angles (Champion and Mitragotri 2006, 2009). Short-rod (aspect ratio = 1.5) mesoporous silica NPs are easily trapped in the liver, whereas long-rod (aspect ratio = 5) silica NPs distribute in the spleen (Huang et al. 2011). Thus, the particles with smaller aspect ratios exhibit more rapid clearance.

In addition to overall shape, the smoothness/roughness of the particle surface also affects the opsonization of the particle and its subsequent uptake by the mononuclear phagocyte system (MPS; Bertrand and Leroux 2012). Particle shape also affects potential toxicities. Titanium dioxide (TiO_2), in its fiber structure >15 μm,

provokes an inflammatory response in alveolar macrophages. Due to alteration of shapes, it becomes difficult for the phagocytic cells to process the NPs, resulting in toxicity by lysosomal disruption (Hamilton et al. 2009).

The surface charge directly affects the interaction of NPs with biological surfaces, cell membranes, and proteins. Charged liposomes (positive or negative) undergo greater opsonization than neutral vesicles do and show greater accumulation in the MPS (Chonn et al. 1991). In mice, undesirable liver uptake has been observed for PEG-oligocholic acid-based micellar NPs with highly positive or highly negative surfaces, whereas liver uptake was low for slightly negatively charged NPs. The NPs had greater accumulation in ovarian tumors (Xiao et al. 2011).

Owing to negative charge on cell surface, the positively charged particles may cause higher nonspecific cellular internalization and relatively shorter circulation half-life. The particles with positive charges are more likely to accumulate within macrophages. The introduction of negative charges into the dextran molecule prolonged its circulation in blood. The derivatization with cationic diethylaminoethyl groups reduced its half-life. The polycationic dextran deposited in the liver more readily than the polyanionic and original dextran macromolecules. Approximately, 10% substitution of dextran with the diethylaminoethyl group was sufficient to enhance the accumulation of dextran in the liver and spleen (Yamaoka et al. 1995).

Conversely, the negatively charged/neutral materials experienced lower nonspecific uptake owing to steric/electrostatic repulsion (Alexis et al. 2008) and resisted the cytotoxic effects. A strong electrostatic barrier sometimes overrides the size or shape factors in exhibiting toxicity (El Badawy et al. 2011).

20.2.2 Route of Exposure

The oral route is most popular among the others for the delivery of drugs due to various advantages like better patient compliance, ease of self-medication, pharmacoeconomic suitability, and painless delivery, which all combine to make this route suitable for chronic therapy (Das and Chaudhury 2011). Apart from these advantages, the oral delivery poses some problems, including the drug interaction with gastrointestinal tract (GIT) content, poor intestinal permeability, and intestinal transit. This can further be complicated by the low solubility and instability of small and large drug molecules. These problems can be resolved by adopting different nanotechnological aspects, where the metallic and polymeric nanocarriers are quite capable of crossing different barriers and enhancing bioavailability of the drug candidate.

Owing to the nature of mucus layer/secretions and turnover, it creates a major barrier for the penetration of NPs across the intestinal tract (Ensign et al. 2012). The mucoadhesive or mucolytic properties of the modified nanostructures could be helpful in crossing the mucosal barriers (Li et al. 2013; Araújo et al. 2014). The mucolytic NPs disrupt the natural mucus barriers, exposing the intestinal surface. This effect enhances the uptake of NPs and also the bacterial attachment and translocation, which may lead to infections (Albanese et al. 1994). Moreover, the cell surface is exposed to the harsh conditions of intestinal tract, leading to their further damage in the absence of mucus layer.

Regardless of the various advantages, the different nanostructures have mild-to-moderate toxicological effects on different tissues and cells. Most of the polymeric

nanocarriers for oral administration contain surfactants for better absorption. In addition, these nanocarriers are often coated with different hydrophilic materials to serve the same purpose. It has been reported that the chronic use of nanostructures containing surfactants can cause disruption to the intestinal epithelium, which further enhances the entry of microorganisms resulting in various pathological changes. The coated biomaterials in the nanostructure can cause structural reorganization of the tight junctions (TJs), leading to disruption of epithelial integrity (Yeh et al. 2011; Sonaje et al. 2012).

The oral toxicity of the NPs may be local or systemic. The local toxicity involves direct interaction of the NPs with the intestinal cells by virtue of their size and charge. In systemic toxicity, all the characteristic features of NPs that influence their translocation and interaction with different tissues must be considered.

Most of the time, the toxic potentials of NPs are neglected when biocompatible and biodegradable materials are used to produce the NPs. The NPs composed of biodegradable materials can also precipitate cellular toxicity due to the intracellular changes caused by their accumulation inside the cells. Moreover, the material properties may change completely upon some chemical modifications. Besides the toxicity of the materials, their degradation products are also another concern.

Furthermore, the reagents used in the production of NPs should be less toxic and the final product must comply with the Food and Drug Administration (FDA) limits (Arora et al. 2012). The long-term use of absorption enhancers can lead to the damage of the intestinal epithelium with the possibility of promoting the passage of pathogens and toxins through the GIT (Fonte et al. 2013). Some biomaterials induce structural reorganization in the TJs or chelate the calcium causing the disruption of TJs (Werle et al. 2009; Yeh et al. 2011), thus enhancing the drug absorption. Often, the toxicity is associated with the materials that are part of the NPs. Nevertheless, the pharmacokinetic properties of a drug or excipient may change considerably following incorporation into nanoparticulate system (Chiu et al. 2009; Baldrick 2010).

Intravenous and subcutaneous injections of nanomaterial-based carriers deliver exogenous NPs into the body for better distribution. However, wider spreading may cause toxicity and undesirable interaction with biological macromolecules. Injected nanomaterials <100 nm are efficiently transported via interstitial flow to the draining lymphatics and lymph nodes. Meanwhile, they reach most of the organs based on their size and surface characteristics. Besides injection, other routes of exposure like nasal and dermal are also common.

It has been shown that metallic NPs <10 nm can penetrate the epidermal layers (Baroli et al. 2007). NPs may pass through the stratum corneum of damaged skin and may induce lung inflammation by stimulating pulmonary epithelial cells to generate proinflammatory cytokines (Nel et al. 2006).

A major challenge in drug delivery is to improve selective targeting and safe strategies, but major caution should be made in a special group of patients like pregnant women, infants, and aged people. For example, studies have shown that NPs can easily cross the placental barrier and induce pregnancy complications (Wick et al. 2010).

Most of the information about kinetics of materials comes from tests of materials in the normal size, and unsurprisingly, there is a lack of data about kinetics of nanosized materials that may have a major role in toxicity (Pourmand and Abdollahi

2012). Therefore, a data bank on biological effects, toxicity, biokinetics, as well as structure and molecular size can assist scientists to predict the toxicity of nanomaterials. The biokinetics of NPs is illustrated in Figure 20.1.

20.2.3 Polymer Characteristics

The polymers offer versatility in both structure and functions due to a wide variety of monomers available, and contribute to the advances of nanodrug delivery systems. Loading capacity as well as controlled delivery of the drug solely depends on the type of polymer used. Out of two kinds of polymers, that is, biodegradable and nonbiodegradable, mainly biodegradable polymers are used in drug delivery. Thus, the chances of toxicological manifestations related to polymeric nanocarriers in drug delivery are lessened. Regardless of this belief, the safety concerns related to polymeric nanocarriers are very important and require further attention.

Of all the polymers, chitosan (CS) is the most widely studied natural polymer for oral drug delivery application. Due to the nontoxic and biocompatible nature, it is approved by the FDA for wound dressing (Baldrick 2010).

Despite extensive investigation with CS, it did not get approval from the FDA for use in any product for the drug delivery, and as a consequence, very few companies are using this material for drug delivery applications (Kean and Thanou 2010).

Toxicity data are still needed to answer some safety concerns in order to include CS as an excipient in new drug formulations. CS is not absorbed by the GIT and is unlikely to show biodistribution, while CS oligosaccharides and its derivatives such as trimethyl chitosan (TMC) are absorbed to some extent (Chae et al. 2005; Zheng et al. 2007). It has been shown that *in vitro* Caco-2 cell or *in vivo* oral absorption of CS derivatives in rats depends on their molecular weight (MW; Chae et al. 2005). Low-MW oligomers (3.8 kDa, 88.4% deacetylation degree [DD]) show relatively higher absorption than high-MW CS (230 kDa, 84.9% DD), which remains almost unabsorbed.

The MW and DD also influence the toxicity of CS. *In vitro* studies have shown that at high DD, the CS toxicity is related to the MW and the polymer concentration; however, at lower DD, the toxicity is less marked and is merely associated with the MW (Schipper et al. 1996, 1999; Agrawal et al. 2014).

Arai et al. (1968) reported an LD_{50} value of 16 g/kg for CS following oral administration to mice. No side effects were reported up to a dose of 4.5 g/day in humans. However, regular intake for 12 weeks produced mild nausea and constipation (Gades and Stern 2003; Baldrick 2010).

The toxicity data regarding the CS derivatives also exist in the literature. Yin et al. (2009) reported the toxicity of TMC–cysteine conjugate (500 kDa) solution. However, the NPs did not produce toxicity. Zheng et al. (2007) noted that TMC NPs could cause light diarrhea at high doses, which can be relieved by discontinuing the administration.

The toxicity of decanoic acid-*g*-oligo CS NPs has also been assessed in rats (Du et al. 2014). The histopathology studies did not exhibit significant differences between the experimental and the control groups. The villi structure of the intestinal epithelium was normal without the presence of inflammatory cells.

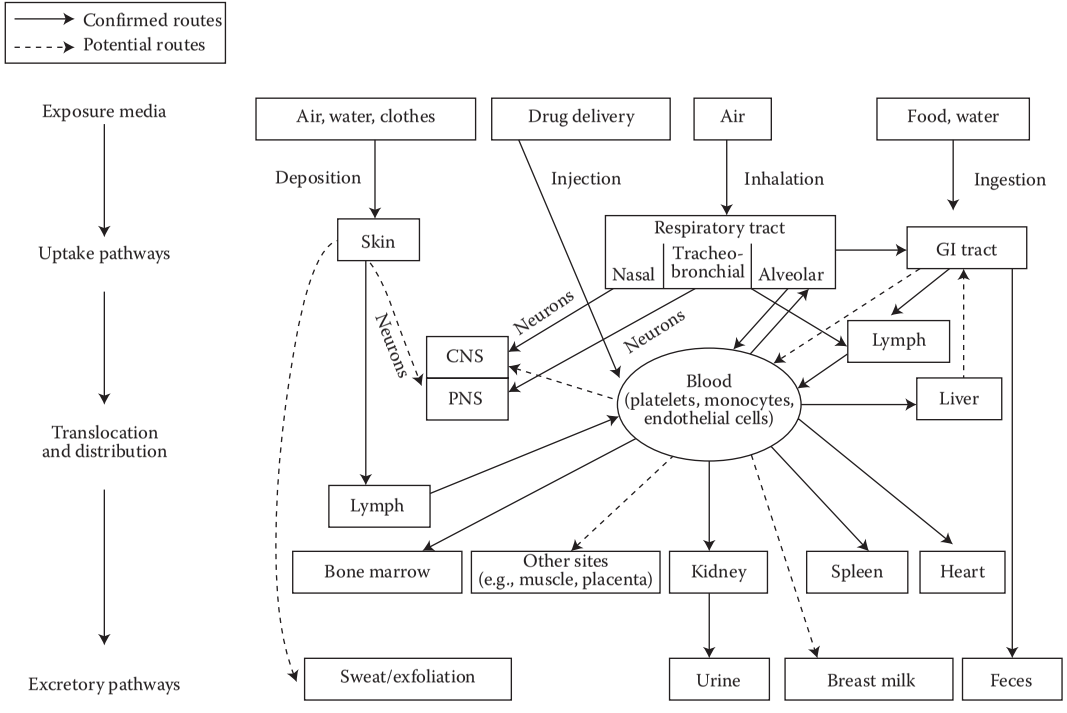

FIGURE 20.1 Biokinetics of nanoparticles. PNS means peripheral nervous system. Although many uptake and translocation routes have been demonstrated, others still are hypothetical and need to be investigated. Translocation rates are largely unknown, as are accumulation and retention in critical target sites and their underlying mechanisms. These, as well as potential adverse effects, largely depend on physicochemical characteristics of the surface and core of nanosize particles (NSPs). Both qualitative and quantitative changes in NSP biokinetics in a diseased or compromised organism also need to be considered. (Reproduced from *Environmental Health Perspectives*, and the material was reproduced from the link: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1257642/.)

Like the natural polymers, the toxicity of synthetic polymers is not addressed in most of the studies. Poly(lactic-co-glycolic acid) (PLGA), a synthetic polymer is used to produce oral controlled release NPs for its biodegradable and biocompatible nature. The safety of PLGA as a drug delivery polymer is supported by a number of studies. In an *in vitro* study on lung epithelial cell line, Yang et al. (2012) concluded that the PLGA polymer is safe for use either alone or in combination with CS. The effect of native CS on cell death was likely more pronounced because it carries highly positive charge. The reduced cytotoxicity of the NPs was probably due to partial neutralization or encapsulation of the positive surface charge of CS by negatively charged low-MW heparin (LMWH) or by PLGA coating. In addition, the low cytotoxicity of CS-PLGA LMWH NPs was supposed to be influenced by the biocompatible and biodegradable nature of PLGA and CS polymers. PLGA NPs (200 nm) presented no toxicity either alone or coated with CS in Caco-2 and HT29 intestinal cell lines (Araújo et al. 2014).

Moreover, Semete et al. (2010a) evaluated the cytokines' expression of CS and PEG-coated PLGA NPs within 24 h of oral and peritoneal administration in Balb/C mice. The expression of proinflammatory cytokines interleukin (IL)-2, IL-6, IL-12p70, and tumor necrosis factor-α (TNF-α) in the plasma and the peritoneal lavages persisted at low concentrations.

The oral toxicity of PLGA NPs stabilized with didodecyldimethylammonium bromide was studied in rats (Bhardwaj et al. 2009). Methylthiazolyldiphenyl-tetrazolium (MTT) and lactate dehydrogenase assays suggested that the cationic surfactant was safe in the cell cultures at concentrations <33 μm. PLGA NPs prepared with this stabilizer were found to be nontoxic on cell lines.

The biodistribution of the PLGA NPs was studied for 7 days after oral administration (Semete et al. 2010b). The cell viability was >75% for PLGA particles, but significantly reduced for zinc oxide particles. *In vivo* toxicity was assessed via histopathological evaluation, and no specific anatomical pathological changes or tissue damage were seen in the tissues of Balb/C mice. The results showed that about 40% of the particles were localized in the liver, 26% in the kidney, and 13% in the brain.

Jain et al. (2011) showed that tamoxifen-encapsulated PLGA NPs (165.58 ± 3.81 nm) could significantly reduce hepatotoxicity than its solution form. The liver section of rats treated with PLGA NPs presented normal histopathology in contrast to tamoxifen solution that presented edema and swelling of hepatocytes, necrosis, hyperplasia of Kupffer cells, and apoptosis.

Polylactic acid (PLA)/cholate NPs were nontoxic at a dose of 75 mg/kg after IV administration to rats (Plard and Bazile 1999). At higher doses, 220 and 440 mg/kg, mortality and marked clinical signs were observed with dose-related hematological changes. Methyl-PEGylated PLA NPs did not show any incidents of lethality and clinical complications even at dose of 440 mg/kg. PEGylation improved the safety profile of PLA/cholate NPs as compared to non-PEGylated NPs. They reasoned that the steric repulsion by the highly dense methyl-PEG chains on the NPs' surface prevented the coagulation cascade and associated toxicity.

Mura et al. (2011) tested lung toxicity of PLGA NPs on human bronchial Calu-3 cells. The positively charged, negatively charged, and neutral NPs were prepared by coating their surface with CS, poloxamer, or poly(vinyl alcohol), respectively.

Regardless of the surface charge, the cytotoxicity of the NPs was very limited, with no signs of inflammatory response.

However, in case of nondegradable block copolymers, there is risk of accumulation in the MPS or other tissues due to lack of elimination. Biodistribution, movement of materials through tissues, phagocytosis, opsonization, and endocytosis of nanosized materials are all likely to have an impact on potential toxicity, which in turn depends on the particle surface charge (Garnett and Kallinteri 2006).

PEG is generally regarded as safe with $LD_{50} > 10$ mg/kg. It has long been used as an excipient in pharmaceutical formulations intended for parenteral, oral, ocular, rectal, and topical use. A little toxicity associated with the exposures of 10 mg/kg for PEG up to 10 kDa are deemed acceptable. However, there are a few long-term toxicological data on PEGs > 10 kDa that are commonly used in the design of NPs. Most of the nonimmunogenic effect of PEG is due to the decrease in opsonin adsorption to the particles, thereby reducing phagocytosis by macrophages of the MPS (Fruijtier-Polloth 2005; Webster et al. 2009).

The hydrophilic natural polymers are currently being investigated as drug delivery carriers due to their nontoxic, biodegradable, and biocompatible nature. In a majority of cases, various polysaccharide derivatives are synthesized for drug delivery applications. The modified polymers are also being screened as potential therapeutic agents. Despite the desirable properties of the native polymers, the same are questionable for their derivatives. Hence, there is also need to consider toxicology of the polysaccharide derivatives, and their NPs since they may cause various interactions with fluids, cells, and tissues, starting at the portal of entry and then via a range of possible pathways toward target organs. At the target organ, the NPs may trigger mediators that may activate inflammatory or immunological responses (Donaldson et al. 2004).

20.2.4 Types of Nanocarriers

20.2.4.1 Dendrimers

Dendrimers are micelle-like NPs that are composed of a hydrophobic core and a hydrophilic shell, constituted by polymeric branches (Svenson and Tomalia 2005). The inherent toxicity of dendrimers in biological system creates a barrier toward extensive pharmaceutical application (Jain et al. 2010). The cationic dendrimer surface interacts with negatively charged biological membranes *in vivo* and exhibits via nanohole generation, thinning, and erosion of membrane. The toxicity in biological system mainly includes hemolytic toxicity, cytotoxicity, and hematological toxicity. The toxicity can be minimized by designing biocompatible dendrimers and masking the peripheral charge by surface modification via PEGylation, acetylation, carbohydrate, and peptide conjugation, or by introducing negative charge such as half-generation dendrimers. Neutral and negatively charged dendrimers do not interact with biological environment, and hence are compatible for clinical applications.

The polymer, poly(amidoamine) or PAA, is a class of dendrimer, which is made up of repetitively branched subunits of amide and amine functionality. The relative ease/low cost of synthesis of PAA dendrimers, along with their biocompatibility, structural control, and functionalizability have made PAA viable candidates for

application in drug delivery (Lee et al. 2005). Initial studies on PAA toxicity showed that PAA was less toxic than related dendrimers of minimal cytotoxicity (Haensler and Szoka Jr 1993; Fischer et al. 2003).

More recently, a series of studies by Mukherjee et al. (2010a,b) have shed some light on the mechanism of PAA cytotoxicity, providing evidence that the dendrimers cause harm to the cell's mitochondria and eventually leading to cell death. It has also been shown that PAA dendrimers cause rupturing of red blood cells (RBCs), or hemolysis (Malik et al. 2000).

Thiagarajan et al. (2013) reported that cationic dendrimers are more toxic than the anionic ones that are tolerated at 10 times higher doses. Moreover, larger dendrimers are more toxic, causing hemobilia and splenomegaly. However, the masking of cationic residues with noncharged groups can improve their safety and uptake by the epithelial cells (Wiwattanapatapee et al. 2000).

To date, a few in-depth studies on the *in vivo* behavior of PAA dendrimers have been carried out. The functionalization of PAA has a dramatic effect on their ability to diffuse in the CNS tissue *in vivo* and penetrate living neurons as shown by intraparenchymal or intraventricular injections in animals. The G4-C12 PAA dendrimer can induce dramatic apoptotic cell death of neurons *in vitro* at a concentration of 100 nM. On the contrary, G4 PAA does not induce apoptotic cell death of neural cells in the submicromolar range of concentration and induces low microglia activation in brain tissue after a week (Albertazzi et al. 2013).

20.2.4.2 Lipid-Based Nanostructures

A number of lipid-based nanocarriers have been designed such as nanostructured lipid carriers (NLCs) and SLNs for the purpose of oral drug delivery. Both systems consist of many components like oil, surfactants, cosurfactants, and cosolvents. These components, especially the surfactants and cosurfactants, can precipitate toxic effects because a large amount of emulsifier is required for their preparation. The materials used for the formulation of SLNs include different triglycerides such as tricaprin, trilaurin, trimyristin, tripalmitin, tristearin, and hard fats such as different grades of Witepsol, Softisan, glyceryl monostearate, glyceryl behenate, stearic acid, palmitic acid, and so on. Regardless of these lipids, the emulsifiers such as soybean lecithin, egg lecithin, and phosphatidylcholine are also used. Emulsifiers are the most important component of SLNs/NLCs and maintain hydrophilic–lipophilic balance (HLB) with lipids to give stability to the formulation. In some cases, when any of the system components got unbalanced, it may cause toxicity. For example, use of Tween 80, a commonly used emulsifier having HLB value very high, can result in loosening of TJs of intestinal epithelium cells (Buyukozturk et al. 2010).

In vivo toxicology of SLNs by Cho et al. (2014) did not reveal any damage to the intestinal epithelium such as villi fusion, occasional epithelial cell shedding, and congestion of the mucosal capillary with blood and focal trauma even 8 h after oral administration.

According to Buyukozturk et al. (2010), the oil structure, surfactant HLB values, and surfactant to oil ratio are important considerations for the safety of the emulsion-based formulations. In case, some of these parameters are imbalanced, toxic effects may occur.

NPs can coexist with surfactants, but this coexistence of NPs and surfactants is likely to give rise to joint toxic effect on biological systems and environment (Wang et al. 2014). Indeed, research results have demonstrated that surfactants embedded into membranes as interstitial ingredients brought about alterations in bilayer structure, as well as dissolving capacity (Schreier et al. 2000). In addition, surfactants absorbed on the surface of NPs may cause surface charges, disparity, and toxicity (Lovern and Klaper 2006; Baalousha 2009), otherwise, they are more likely to decrease toxicity effects due to inhibiting interactions between NPs and bacteria by means of steric hindrance and charge repulsion (Zhang et al. 2007). Thus, safety studies of all the materials and lipid-based nanostructures are essential prerequisite before their clinical applications.

20.2.4.3 Carbon Nanotubes

Carbon nanotubes (CNTs) are cylindrical structures formed by rolling of single layer (single-walled CNT [SWCNTs]) or multiple layers (multiple-walled CNT [MWCNTs]) of graphene sheets with diameters of 1–2 nm and lengths of 0.05–1 μm (Foldvari and Bagonluri 2008). The allotrope of carbon consists of 60 carbon atoms joined together to form a cage-like structure. C60 is soluble in aromatic solvents (e.g., toluene or benzene), but insoluble in water and alcohol. However, C60 can be functionalized with —OH, —COOH, or $—NH_2$ to increase its hydrophilicity.

The cylindrical structures are capped at the ends by carbon networks. CNTs are being explored as drug nanocarriers due to their high surface area, conductivity, high tensile strength, and potential higher absorption capabilities (Beg et al. 2011). The hollow monolithic structure of CNT allows the incorporation of drug molecules for controlled and site-specific delivery (Heister et al. 2009). Moreover, the outer surface of CNTs can be functionalized to enhance their biocompatibility and biodegradability (Beg et al. 2011). There have been several toxicological studies after oral administration of CNTs. However, the study reports are contradictory to each other. Some workers reported acute toxicity and genotoxicity with CNTs, while the others reported no toxic influence of the CNTs. It was previously shown that CNT had some immunological reactions of CNTs. Later on, the effects were ascribed to the metallic impurities and contaminants present in the CNTs (Pulskamp et al. 2007).

The high purity and well-dispersed sample of SWCNTs (3.0 ± 1.1 nm, length <1.2 μm) did not exhibit any genotoxic effects in both *in vitro* and *in vivo* experiments at a dose of 60 and 200 mg/kg BW (Naya et al. 2011). Single-dose genotoxicity study in Fischer 344 rats revealed that the nanotubes (0.9–1.7 nm) elevated the levels of 8-oxo-7,8-dihydro-2′-deoxyguanosine in lungs and liver at doses of 0.064 and 0.64 mg/kg BW. The nanotubes caused oxidative damage to DNA in liver and lung cells after oral administration (Folkmann et al. 2009). A single bolus dose of ultrashort and full-length SWCNTs (diameter = 1 nm length = 2 μm–20 nm; 1000 mg/kg BW) in Swiss mice did not produce causalities and abnormalities. No acute oral toxicity was observed regardless of the length, surface area, and surface interactions (Kolosnjaj-Tabi et al. 2010).

Studies on carbon nanomaterials have indicated the potential neurotoxic effects after inhalation or systemic exposure. Oberdörster and coworkers (2004) showed that inhalation of elemental ^{13}C particles (36 nm) following whole-body exposure

for a period of 6 h led to a significant and persistent increase in the accumulation of ^{13}C NPs in the rat's olfactory bulb, and the NP concentration gradually increased. However, different shapes of carbon nanomaterials may elicit different neuronal toxicity.

More specifically, pure graphene exhibits less toxicity than highly purified SWCNTs in a concentration-dependent manner after 24-h exposure of PC12 cells, involving the apoptosis pathway (Zhang et al. 2010b).

CNTs with surface coating of PEG are less toxic on mitochondrial function and membrane integrity than uncoated CNTs. A study has shown that oxidative stress is involved in this toxic pathway, with surface coating playing an important role (Zhang et al. 2011). It has been reported that 14-nm carbon black particles may translocate to the olfactory bulb through olfactory neurons, resulting in the activation of microglial cells, which induces proinflammatory cytokines and chemokines, suggesting an inflammatory response (Shwe et al. 2006). Further *in vivo* studies are needed to understand the effect of surface coating on the biocompatibility of these carbon-based nanomaterials prior to use in humans.

20.2.4.4 Metal NPs

The most widely studied metal NPs include AuNPs, and superparamagnetic iron oxides' (Fe_2O_3 or Fe_3O_4) NPs (SPIONs). However, the use of these NPs as oral delivery systems is very limited due to crisis in toxicological studies (Li and Chen 2011).

A report by Hillyer and Albrecht (2001) indicated that 4-nm AuNPs could cross the GIT more readily, resulting in higher accumulation in kidney, liver, spleen, lungs, and brain of mice compared to the particles of 10–58 nm size. Pokharkar et al. (2009) did not find any changes in clinical signs, body weight, food consumption rate, hematological parameters, organ weights, and histopathological observation for CS-coated AuNPs in rats after 28 days of oral administration. Moreover, the LD_{50} was >2000 mg/kg BW. Zhang et al. (2010a) compared the toxicity of different oral doses (137.5–2200 μg/kg) of AuNPs of 13.5 nm size. The particles were almost nontoxic at lower doses. However, a reduced RBC count was noticed at higher doses, with higher accumulation in spleen. Thus, the factors such as size, surface coating, and the dose are among important considerations in developing oral AuNPs formulations.

The SPIONs are composed of Fe_3O_4 (magnetite) or Fe_2O_3 (maghemite) core. They are specifically used for brain imaging or brain-targeted drug/gene delivery due to their ability to cross the blood–brain barrier (BBB; Kong et al. 2012). Despite their desirable traits, the *in vivo* and *in vitro* toxicity data are of great concern before clinical application. SPIONs can interfere with gene expression, actin modulation, cell cycle regulation, and signaling pathways, and may lead to excessive ROS generation and disruption of iron homeostasis (Singh et al. 2010).

According to Wang et al. (2009), the transport of submicron level Fe_3O_4 NPs to the brain via the olfactory nerve pathway may cause oxidative stress-related damage in brain. They also demonstrated size-dependent effect on iron deposition in different brain regions after single intranasal exposure of 21-nm and 280-nm Fe_2O_3 NPs in mice (Wang et al. 2008). The iron content in olfactory bulb, hippocampus, cerebral cortex, and cerebellum to the brainstem significantly increased after administration

of smaller particles. However, the iron deposition was significant only in olfactory bulb and hippocampus for larger particles. Even after 30 days, the iron content in these regions was lower than that in mice treated with 21-nm Fe_2O_3 NPs. The brain iron accumulation is associated with oxidative stress induced by the formation of the highly reactive *OH via the Fenton reaction (Kim et al. 2000; Castellani et al. 2007).

The generation of ROS is a well-established paradigm to explain the toxic effects of NPs. Wu et al. (2013) focused on the neurotoxicity of iron oxide NPs in the rat brain *in vivo*. Overall, the number of studies regarding the toxicity of metallic NPs is very limited. Most of the studies focused on the biodistribution of the NPs. However, the study regarding interaction of NPs with the tissues is lacking. Hence, the safety of metallic NPs must be ensured.

20.3 EXPERIMENTAL NANOTOXICITY ON DIFFERENT PHYSIOLOGICAL SYSTEMS

NPs enter the human body through various routes, including respiratory tract, GIT, skin contact, IV injection, and implantation. Following absorption, the NPs are carried to distal organs by the bloodstream and the lymphatic system. The possible nanotoxicity to physiological systems is represented in Figure 20.2.

20.3.1 Circulatory System

NPs are transported to distal organs through the blood. During translocation, NPs alter fluid dynamics of blood, affect vascular walls, and adhere to the blood vessel surfaces due to nonspecific van der Waals, electrostatic, and steric interactions (Decuzzi et al. 2005).

This trend may relate to physical properties of the NPs like size and shape. Oblate-shaped NPs adhered to the surface of blood vessels greater than spherical NPs of the same volume (Decuzzi and Ferrari 2006). In blood, the original properties of the NPs are changed by proteins that form a protein corona on their surface (Demir et al. 2011). This protein corona influences *in vivo* behavior of NPs such as cell uptake and biocompatibility. Protein adsorption also helps in better dispersion of NPs and causes a higher cellular accumulation of NPs.

NPs are harmful to the circulatory system also. After inhalation, the NPs may stimulate the generation of oxidative stress in the lungs of animal models and lead to the release of proinflammatory mediators and coagulation factors, which are then transmitted to the circulation, leading to cardiovascular lesions, including platelet aggregation, thrombosis, and cardiovascular malfunction (Donaldson et al. 2001).

NPs can induce circulation toxicity after entrance via inhalation as follows. The macrophages located in the alveolar epithelium release cytokines after NPs' uptake. These cytokines migrate across endothelium of the blood vessel and stimulate cardiovascular lesions. Moreover, the NPs migrated across the interstitium are picked up by endothelium macrophages. Consequently, cytokines are released into blood and aggravate cardiovascular lesions. The particles escaping interstitium and endothelium uptake are taken up by blood cells such as platelets and stimulate cardiovascular lesions. The events are described in Figure 20.3.

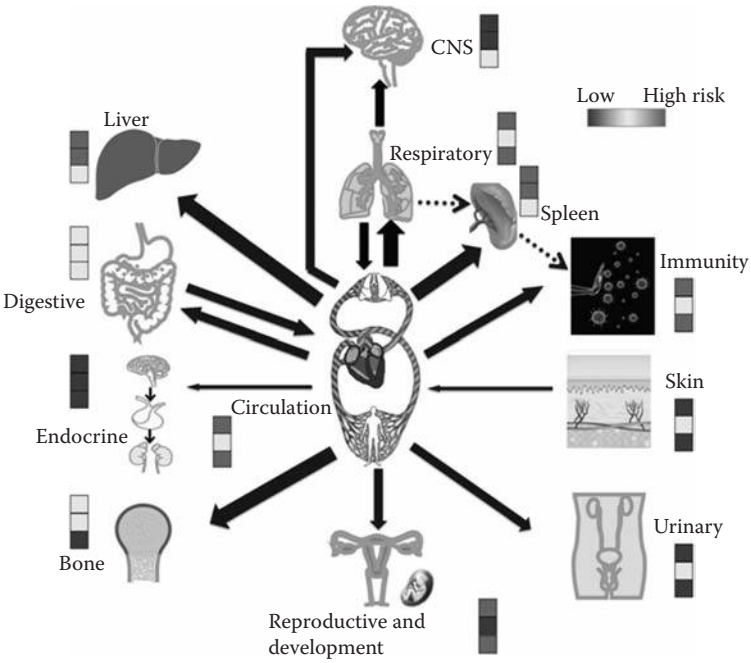

FIGURE 20.2 A working model indicating possible nanotoxicity to physiological systems. In the three-frame bar, three frames (from upper to lower) represent the probability of nanoparticle accumulation in organs or systems, self-repair capability (including the inclination of nanoparticle degradation to facilitate the excretion) of the system, and the observed toxicity from the available literature. The nanoparticle accumulation, self-repair capability, and observed toxicity are intermediate between low and high levels for the digestive system. These effects appear in high-low-high order for reproductive system, respectively. For the rest of the organs or systems, open boxes indicate intermediate level of effects. The closed boxes indicate high level of effects for liver, spleen, circulation, immunity, and respiratory systems and low level of effects for endocrine, bone, CNS, skin, and urinary systems. The scale bar indicates low (left), intermediate (middle), and high (right) levels. These scales are only based on available data and are not conclusive because of differences in dose, nanoparticle preparation, and animal models. Arrows show the direction of nanoparticle translocation. The width of the lines indicates the readiness of nanoparticle translocation. Dashed lines show the reported cross-system effects. (Zhang, Y. et al. 2014. Perturbation of physiological systems by nanoparticles. *Chem Soc Rev* 43:3762–809. Reproduced by permission of The Royal Society of Chemistry.)

In blood, NPs also activate some coagulation pathways. MWCNTs with different habits like pristine, carboxylated, and amidated damage endothelial cell of blood vessel and trigger coagulation *in vivo*. *In vitro*, they exhibit obvious procoagulant activity with activated partial thromboplastin time (aPTT) assays (Burke et al. 2011). MWCNTs may activate both intrinsic and extrinsic pathways of coagulation via factor IX- and factor XII-dependent ways and stimulate thrombosis. Carbon NPs (MWCNTs, SWCNTs, and mixtures thereof) (Radomski et al. 2005) can enhance platelet aggregation and contribute to the vascular thrombosis. Though the thrombus

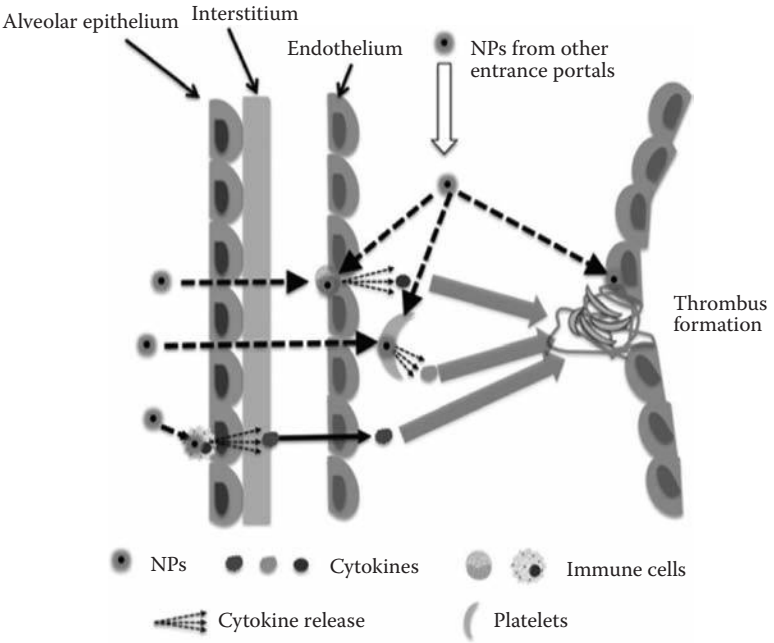

FIGURE 20.3 Nanoparticle-induced circulation toxicity after inhalation. (Zhang, Y. et al. 2014. Perturbation of physiological systems by nanoparticles. *Chem Soc Rev* 43:3762–809. Reproduced by permission of The Royal Society of Chemistry.)

sizes are dose dependent (Hoet et al. 2004; Vermylen et al. 2005), these effects may cause significant risks in populations for atherothrombosis.

20.3.2 Bone Marrow

Under certain pathological conditions, the liver, thymus, and spleen may resume hematopoietic functions, causing their pathological enlargement. This may impair production of blood cells by affecting the hematopoietic stem cell functions, immune functioning, and lead to diseases like leucopenia, thrombocytopenia, neutropenia, and anemia.

Like the liver and spleen, bone marrow is one of the primary organs of the reticuloendothelial system where the production and maturation of most blood cells occur. Thus, the access of NPs to bone marrow is quite expected. After oral administration into mice, polystyrene NPs were detected in bone marrow. Polystyrene microparticles in the size range 50 nm–3 μ were fed by gavage to female Sprague Dawley rats daily for 10 days at a dose of 1.25 mg/kg. The extent of absorption of 50-nm particles under the conditions of these experiments was 34% and of the 100-nm particles was 26%. Particles larger than 100 nm did not reach the bone marrow (Jani et al. 1990). Hence, NPs are currently being investigated for targeting bone marrow for drug delivery.

The adverse hematopoietic effects of particles also depend on the route of administration. After 4 weeks, the inhalation of magnetic NPs decreased the mean corpuscular volume and hemoglobin content, two indicators of impaired erythrocyte

function. Inhaled NPs also decreased the platelets production, increased white blood cell (WBC) count in the bone marrow, and induced extramedullary hematopoiesis in the mouse spleen, which was indicative of pathological conditions such as anemia (Kwon et al. 2009).

20.3.3 Reproductive Systems

The reproductive nanotoxicity takes into account the adverse effects on germ cells, physiological structure and function, fertility, and their effects on the offspring. The AuNPs (9 nm) penetrated the heads and tails of healthy male human sperm cells and caused 25% of sperm cells to become immotile at a concentration of 44 mg/mL (Wiwanitkit et al. 2009).

Repeated IV injection of water-soluble MWCNTs into male mice caused reversible testicular damage without affecting fertility (Bai et al. 2010). Nanotubes accumulated in the testes generated oxidative stress and lowered the thickness of seminiferous epithelium at day 15, but recovered after 60 and 90 days. The quantity, quality, and integrity of the sperm and the levels of sex hormone remained undisturbed throughout the entire study period.

After tail vein injection to pregnant Sprague Dawley rats, [^{14}C]C60 NPs (~0.3 mg/kg BW) cross the placenta and is transmitted to offspring via the dam's milk and subsequently drained into blood (Sumner et al. 2010). Some colloidal Au particles (5 and 30 nm) are transferred to the fetus 1 h after IV injection at gestational day 19. Small AuNPs exhibited a slightly higher transfer rate than 30-nm NPs (Takahashi and Matsuoka 1981). The NPs may be transferred from placenta to the fetus, where they may exhibit potential developmental toxicity. In one study, pregnant Slc mice were injected intraperitoneally (IP) with C60 NPs on gestational day 10 and the embryos were examined 18 h after injection (Tsuchiya et al. 1996).

At a dose of 50 mg/kg, the NPs distributed into the yolk sac and embryos, and half of the embryos deformed in the head and tail regions. At a dose of 25 mg/kg, abnormal embryos were less frequent; however, all embryos died at a dose of 137 mg/kg. It was speculated that C60 NPs caused severe dysfunction of the yolk sac and embryonic morphogenesis.

20.3.4 Gastrointestinal Tract

Upon oral administration, NPs have only transient contact with the oral cavity, pharynx, and esophagus. A majority of them are accumulated in the stomach and intestines and the unabsorbed fraction is quickly eliminated thorough feces. Due to protective mucous layer and the tight epithelial junctions, the rate of absorption of NPs from GIT is much lower than other routes. Under certain pathological conditions, however, the integrity or function of one or more GI layers is compromised and the layers become permeable, causing disorders such as inflammatory bowel disease.

The NPs retained in the GIT may adversely affect its structure and function. Recently, a pH-responsive NP system shelled with CS has been found to effectively increase the oral absorption of insulin and produce a hypoglycemic effect, presumably due to the CS-mediated TJ opening (Sonaje et al. 2011).

Using *in vitro* model of the intestinal epithelium and *in vivo* chicken intestinal loop model, acute and chronic oral exposures to polystyrene NPs were studied (Mahler et al. 2012). Intestinal cells showed increased iron transport at high doses due to disruption of cell membrane by the NPs. Chickens acutely exposed to carboxylated particles of 50-nm sizes had lower iron absorption than unexposed or chronically exposed birds. Chronic exposure possibly caused remodeling of the intestinal villi and increased the surface area available for iron absorption. In addition to potential impact of NPs on nutrient absorption, this report emphasized the complexity of interactions between NPs and GI tract.

20.3.5 Urinary Nanotoxicity

Since NPs readily accumulate in kidney in addition to the reticuloendothelial system, their urinary toxicity is a prioritized concern. Further, the kidney is an important organ for the elimination of NPs (Li and Huang 2008).

Larger NPs are primarily localized in the liver and spleen. However, small particles (~5–10 nm) may pass glomerular barriers (glomerular endothelial cells

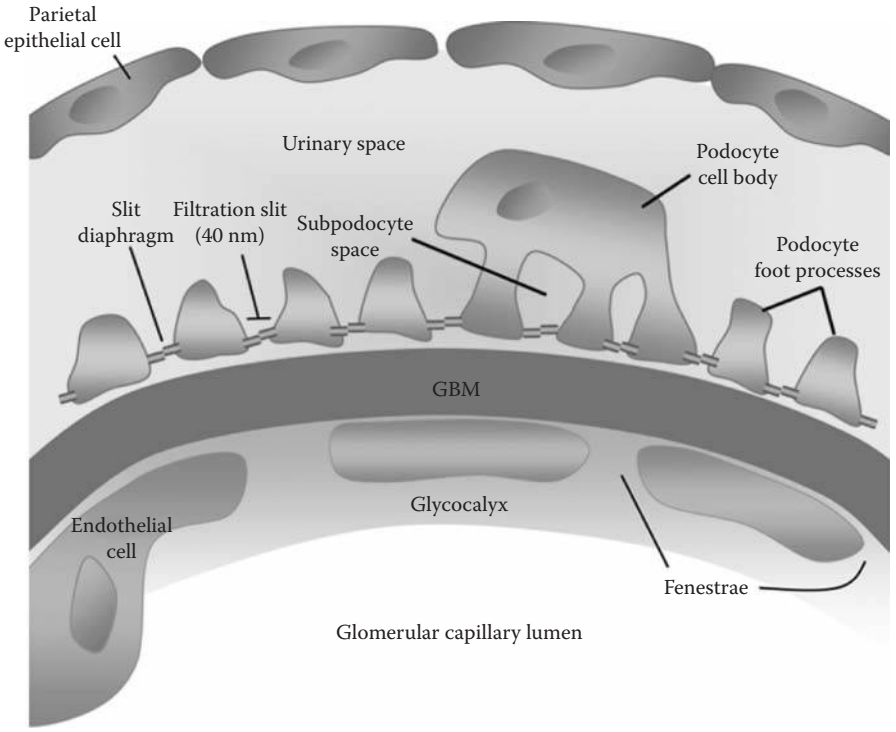

FIGURE 20.4 Glomerular capillary wall. (Reprinted from *Am J Kidney Dis*, 58, Jefferson, J.A. et al., Podocyte disorders: Core curriculum, 666–77, Copyright 2011, with permission from Elsevier.)

fenestrate, 80–100-nm wide pores, glomerular basement membrane, and podocytes) and excrete rapidly in urine (Longmire et al. 2008; Figure 20.4).

Larger NPs that diffuse through the glomerular endothelial cell fenestrate, 80–100-nm wide pores are further prevented by the glomerular basement membrane and podocyte foot processes. The basement membrane, together with the podocyte foot processes, imposes an apparent cutoff size of ~10 nm or MW of 30–50 kDa. However, some SWCNTs can penetrate the physical barriers and excrete in mice urine. SWCNTs (0.8–1.2 nm in diameters and 100–500 nm in length) are cleared intact by glomerular filtration, with partial tubular reabsorption and transient translocation into proximal tubular cell nuclei ($t_{1/2}$ ~ 6 min) after IV injection. The threshold MW for the glomerular filtration of polymers lies in the range of 30–50 kDa and depends on charge, molecular conformation, and deformation ability. Because of high aspect ratio (d ~ 1 nm, $100 \leq L \leq 500$ nm), negative charge, and high MW (150–750 kDa), the construct largely exceeds structural sizes of the glomerular pores (at least in the longitudinal dimension). The renal elimination of ~65% of the recovered construct was observed with ~15% of the construct undergoing passive reabsorption within the tubules at 20 min postinjection. This can be regarded as an exceptional case, probably due to their needle-like shape (Ruggiero et al. 2010).

Studies indicated that kidney is relatively insensitive to the adverse effects of NPs. IP and IV injection of *N*-octyl-*O*-sulfate chitosan (NOSC) NPs into mice led to systemic toxicity, without any histopathological changes in the kidneys (Zhang et al. 2008). The LD_{50} values of NOSC were found to be 102.59 and 130.53 mg/kg, respectively, after IV and IP administration. Almost 75% of the dose of tritium-labeled NOSC (13.44 mg/kg) was excreted in urine over 7 days. NOSC was predominantly excreted through urine, rather than bile or feces.

20.3.6 Central Nervous System

The BBB and blood–cerebrospinal fluid barrier afford protection to the microenvironment of human CNS from hazardous xenobiotics, however, make CNS delivery of therapeutics difficult. Small particles could be advantageous as therapeutic carriers for the treatment of CNS diseases (Bharali et al. 2005) and raises concerns regarding their possible unwanted toxic effects.

The NPs can enter the CNS at least by three distinct ways. Firstly, NPs can penetrate the BBB without damaging its integrity. PEG-grafted CS copolymer (Veiseh et al. 2009) and silica-coated magnetic NPs (Kim et al. 2006) penetrated the BBB without affecting its functions. This mode of penetration is ideal for the therapy of CNS diseases. A biodistribution study suggested that only 0.3% of AuNPs (10 nm) were distributed in rat brain after 24 h, but no Au particles with diameters of 50, 100, or 250 nm were detected after injection into their tail vein (De Jong et al. 2008). Thus, the ability to cross the BBB probably depends on particle size.

The disruption of the BBB integrity is the second option for NPs' penetration. Direct disruption of the cell membrane caused by NPs will allow their entry into the brain. Breakdown of the BBB enables the passage of various serum components, including proteins and other toxic substances into brain microfluid environment. Polysorbate 80-coated poly(*n*-butylcyanoacrylate) (PBCA) NPs are able to cross the

BBB *in vitro* and *in vivo* (Rempe et al. 2011). The disruption of the barrier by polysorbate 80-coated PBCA NPs became reversible after 4 h. Instead of incorporating therapeutic agents into the NP, the drugs may cross the BBB with simultaneous administration of the PBCA NPs.

Lastly, the NPs can translocate to the brain via olfactory nerve pathway, bypassing the BBB. The accumulation of NPs in the cerebral compartment generates oxidative stress and inflammation, causing damage to brain nerve cells. CNS damage may be more severe than other tissues due to weak antioxidant and the self-regenerative ability of neurons. After penetration, the NPs induce morphological changes of nerve cells in cerebral cortex, hippocampus, cerebellum, thalamus, hypothalamus, and brainstem, and cause damage to myelinated fibers as well as the degeneration of nerve cells (Sharma 2007).

20.3.7 Hepatotoxicity

Liver is the major organ for accumulation of NPs. In liver, both hepatocytes and Kupffer cells selectively take up surface-modified NPs. NPs can be excreted from liver via biliary pathway. Eleven days after exposure, approximately 5% of total hydroxylated SWCNTs administered IP were excreted in feces (Wang et al. 2004).

It possesses self-protecting capability due to its antioxidant system and various metabolizing enzymes. However, the long-term retention of NPs may increase the risk of hepatotoxicity (Yang et al. 2008). The prolonged retention of TiO_2 and CNT NPs caused injury to hepatocytes as was evident by histopathologic examination and abnormal serum levels of liver function indicators such as aspartate aminotransferase and alanine aminotransferase (Liang et al. 2008).

The hepatocytes cytoplasmic degeneration and nuclear destruction suggest that AuNPs interact with the proteins and enzymes of hepatic tissues, interferes with antioxidant defense mechanism, and leads to ROS generation. This in turn induces stress in the hepatocytes resulting in atrophy and necrosis (Abdelhalim and Jarrar 2012).

Injection of PEGylated AuNPs (15 nm) have been found to cause severe hepatic cell damage, acute inflammation, higher apoptosis, and ROS production in the livers of mice, which were on methionine- and choline-deficient (MCD) diet for 4 weeks. AuNPs demonstrated toxicity in a stressed liver environment by stimulating inflammatory response and accelerating stress-induced apoptosis (Hwang et al. 2012). Other effects of NPs on bile secretion, glucose and fatty acids synthesis, and blood iron content are largely unknown.

20.4 CONCLUSION

Despite a significant advancement on research works on nanoparticulate drug delivery systems, the number of marketed products is minimal. One possible reason could be the lack of toxicity and safety information related to different nanoparticulate systems that are needed to surpass the regulatory requirements.

Each nanosystem is unique based on material characteristics, and thus requires a case-specific toxicity study. A basic and conceptual understanding of the interactions of the nanosystems with the biological systems is needed in order to have safe and

effective nanosystems for improved drug delivery applications. Overall, the information regarding the toxicology of the NPs is still very limited, which makes it difficult to draw any conclusions regarding the safety and efficacy of nanoparticulate drug delivery systems.

There is an urgent need to understand the potential toxicities of nanomaterials, which would provide useful information to develop safer and more efficient nanoformulations. The safety profiles of the materials used in the nanosystems cannot be directly translated to the final NPs. The size, charge, and surface chemistries of the NPs also influence the biokinetics and toxicity of the systems. Thus, detailed toxicity-safety profiles could help in fulfilling the stringent requirements by the regulatory authorities and will give faster acceptance.

A collaborative research between formulation development scientists and toxicologists is an hour of need to realize the benefits of nanotechnology in human health.

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